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Na^+/H^+ exchange inhibition reduces hypertrophy and heart failure after myocardial infarction in rats

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Kusumoto, Keiji, James V. Haist, and Morris Karmazyn. Na^+/H^+ exchange inhibition reduces hypertrophy and heart failure after myocardial infarction in rats. *Am J Physiol Heart Circ Physiol* 280: H738–H745, 2001.—We investigated the effect of sodium/hydrogen exchange inhibition (NHE-1) on hypertrophy and heart failure after coronary artery ligation (CAL) in the rat. Animals were subjected to occlusion (or sham) of the left main coronary artery and immediately administered a control diet or one consisting of the NHE-1 inhibitor cariporide for 13–15 wk. Hearts were separated by small [≤30% of left ventricle (LV)] and large (>30% of LV) infarcts. CAL depressed change in left ventricular increase in pressure over time (LV +dP/d*t*) in small and large infarct groups by 18.8% ($P < 0.05$) and 34% ($P < 0.01$), respectively, whereas comparative values for the cariporide groups were 8.7% (not significant) and 23.1% ($P < 0.01$), respectively. LV end-diastolic pressure was increased by 1,225% in the control large infarct group but was significantly reduced to 447% with cariporide. Cariporide also significantly reduced the degree of LV dilation in animals with large infarcts. Hypertrophy, defined by tissue weights and cell size, was reduced by cariporide, and shortening of surviving myocytes was preserved. Infarct sizes were unaffected by cariporide, and the drug had no influence on either blood pressure or the depressed inotropic response of infarcted hearts to dobutamine. These results suggest an important role for NHE-1 in the progression of heart failure after myocardial infarction.

cariporide; cellular remodeling

CONGESTIVE HEART FAILURE is an important and rapidly expanding clinical problem with 400,000 new cases diagnosed each year in the United States. Hypertrophy is an early maladaptive response in the heart failure process (14), and its attenuation is therefore, a principal therapeutic goal (3). Sodium/hydrogen exchange (NHE) is a major proton extrusion pathway, critical for intracellular pH (pH_i) regulation. However, in addition to its role in pH_i regulation, the antiporter also contributes to myocardial injury produced by both ischemia and reperfusion. Inhibitors of NHE, particularly newly developed NHE-1-specific inhibitors such as cariporide, and other agents, protect the ischemic myocardium in a wide variety of animal species (1, 6, 7, 12, 19, 22 and reviewed in 13). Although predominant attention is related to cardioprotection, recent evidence suggests NHE-1 may also be important in cardiac cell growth (2, 4, 9, 11, 30); and the activity of the antiporter is augmented by hypertrophic factors such as α_1-adrenergic activation (32), endothelin-1 (15), and angiotensin II (8, 20). This led to the hypothesis that NHE-1 is the downstream mediator for at least some of these factors and that inhibiting NHE-1 would limit the cellular hypertrophy and, potentially, the heart failure process (4). NHE-1 inhibition could limit postinfarction responses as a result of infarct size reduction (29). We have recently shown that dietary administration of the NHE-1-specific inhibitor cariporide 1 wk before coronary artery occlusion attenuates early (1 wk) left ventricular (LV) myocyte hypertrophy and early hemodynamic abnormalities (33), in the absence of any infarct-reducing effects. The potential role of NHE-1 in chronic postinfarction responses is not known with certainty, particularly with respect to its direct influence independent of infarct size attenuation. Accordingly, the present study was carried out to assess the effect of cariporide in a chronic model of heart failure when administered immediately after infarction produced by sustained coronary artery occlusion. We assessed both in vivo hemodynamic responses and ex vivo myocyte characteristics after treatments.

METHODS

Experimental protocol. Male Sprague-Dawley rats weighing 275–300 g (Charles River; St. Constant, Quebec, Canada) were randomly assigned to four groups: sham surgery control diet; sham surgery cariporide diet (containing 3,000 parts per million of cariporide); coronary artery ligation (CAL) control diet; or CAL cariporide diet. Rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg), intubated, and artificially ventilated (10 ml/kg, 70 strokes/min) by using a rodent respirator (model 683, Harvard Apparatus). A lead II electrocardiogram was recorded by using a Grass electrocardiogram amplifier (model 7P6D, Grass Medical Instruments; Quincy, MA). A left thoracotomy was performed, and the heart was gently exposed. During surgery, rectal temperature was kept at 37°C. To induce myocardial infarction, the left main coronary artery was ligated ~3 mm from its origin

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by using a firmly tied silk suture (5-0). Ischemia was confirmed by changes in the S-T segment of the electrocardiogram and by visible blanching of the heart muscle. If both parameters did not alter after ligation, reocclusion was immediately performed. For sham operation, the ligature was placed in an identical fashion but not tied. The incidence of ventricular fibrillation was noted for the first 20 min after ligation, and, if necessary, defibrillation was attempted by gently touching the LV with a wet cotton-tipped applicator. The chest was then closed in three layers (ribs, muscle, and skin), and the animal was allowed to recover. For cariporide treatment, an initial administration of the drug (30 mg/kg ip) was made immediately after ligation or sham procedure and again 8 h later. Regular eating generally resumed 10–12 h after surgery. Identical saline injections were made for the normal diet groups. Rats were given free access to rat chow and water from the first day of surgery and for the duration of the study.

Measurement of hemodynamic parameters. In vivo hemodynamic measurements were performed under anesthesia with pentobarbital sodium (40–50 mg/kg ip) 3 mo after surgery. A catheter (3-Fr, Atom Medical) connected to a pressure amplifier (7P1G, Grass Medical Instruments) was inserted into the right carotid artery and advanced into the LV to measure simultaneous changes in pressure. A catheter (PE-50, Clay Adams) was also inserted into the femoral artery to measure systemic blood pressure. The first derivative of LV pressure was simultaneously monitored by using a Grass 7P20C differentiator amplifier. Heart rate was obtained from the LV pressure recordings by using a Grass 7P44B tachometer.

Measurement of myocardial infarct size. After hemodynamic measurements were performed, the LV and right ventricle (RV) were weighed, and the LV was fixed in 10% buffered formalin (pH 7.4). Infarct size was determined as described recently (33). The fixed LV was cut transversely from apex to base into ~2-mm slices. These slices were embedded in paraffin, and a thin section (5 μm) was obtained from each slice, mounted on glass slides, stained with picrosirius red, and photographed. Photographs were magnified. The epicardium and endocardium circumferences and infarcted portion were measured by a planimeter. Infarct size was calculated by dividing the sum of the infarcted portion by that of the LV circumference. From these measurements, rats were placed into two subgroups: small (≤30% of LV) and moderate to large (>30% of LV) infarct sizes.

Assessment of myocyte characteristics and function. For these experiments, rats were not subjected to either hemodynamic assessments or infarct size determination but were anesthetized with pentobarbital (50 mg/kg ip). The hearts were immediately removed and placed in ice-cold Ca^{2+}-free HEPES solution containing (in mM) 135 NaCl, 5.4 KCl, 1.0 $MgCl_2$, 0.33 NaH_2PO_4, 10 HEPES, and 10 glucose (pH 7.2, 4°C, bubbled with 100% O_2) and then rapidly mounted by the aorta on a Langendorff perfusion system. Retrograde perfusion was initiated with Ca^{2+}-free HEPES solution (37°C) for 5 min. The perfusate was then switched to Ca^{2+}-free HEPES solution containing 1.8 mg/ml of collagenase (Type II, Washington Biochem), 0.1 mg/ml of protease (Type XIV, Sigma), and 0.5 mg/ml of BSA (Sigma) for 12 min followed by perfusion with HEPES buffer containing 0.2 mM $CaCl_2$ (37°C) for 5 min. The heart was then removed from the Langendorff system, and, if necessary, the infarct area was discarded and tissues were cut into small pieces and shaken for 15 min in a water bath at 37°C. Cardiac myocytes were then filtered through a 210 nylon mesh and gently centrifuged at 500 *g* for 45 s. The supernatant was aspirated, 35 ml of HEPES solution containing 0.5 mM $CaCl_2$ were added, and the suspension was left to stand for 10 min, after which the supernatant was aspirated again. Cardiac myocytes were finally suspended in 10–30 ml of HEPES solution containing 1 mM $CaCl_2$ to produce a concentration of ~100,000 cells/ml. The percentage of rod-shaped cells was determined for each isolation and averaged ~80%, irrespective of treatment.

Table 1. *Infarct sizes, body weight, and ventricular weight in normal and cariporide-treated rats with or without myocardial infarction*

	Sham	Infarcted	
		Small	Large
n			
Normal diet	18	13	12
Cariporide diet	15	14	10
Infarct size, %			
Normal diet		24±2	36±1
Cariporide diet		22±1	35±1
Body weight, g			
Normal diet	539±2	540±12	512±13
Cariporide diet	514±11	512±23	503±14
LV weight, mg			
Normal diet	1,017±21	1,135±22†	1,094±36
Cariporide diet	987±21	990±26	1,011±31
RV weight, mg			
Normal diet	237±8	244±9	397±29†
Cariporide diet	220±7	224±7	263±17§
LV/Body weight ratio, mg/g			
Normal diet	1.89±0.03	2.11±0.05†	2.13±0.06†
Cariporide diet	1.85±0.03	1.93±0.04*‡	2.00±0.05*
RV/Body weight ratio, mg/g			
Normal diet	0.44±0.01	0.45±0.02	0.78±0.06†
Cariporide diet	0.43±0.01	0.44±0.01	0.52±0.03*§

Values are means ± SE; *n*, number of rats. LV, left ventricle; RV, right ventricle. *P < 0.05 from respective sham; †P < 0.01 from respective sham; ‡P < 0.05, §P < 0.01 from respective normal diet.

An aliquot of cells was mounted on the thermoregulated (35°C) stage of an inverted microscope (Zeiss Axiovert 65) for 5 min and superfused with HEPES solution containing 1 mM $CaCl_2$ at a rate of 1 ml/min. The cell image was monitored on a video screen, and cell length and width were measured by using an Argus 10 image processor (Hamamatsu, Japan). Cell area was calculated by the multiple of cell length and width. Fifty cells were randomly selected for measurement from each heart and the mean value was used as the individual value for each heart (n = 1). Field stimulation (0.5 Hz, 20–25 V, 5 ms duration) with bipolar platinum electrodes was then initiated, and cell shortening was recorded on a medical-grade tape by using a S-VHS videotape recorder (BR-S601MU, JVC) and was analyzed by using an Argus 10 image processor. Cell shortening was expressed as the percent reduction of cell length from diastolic length. At least 10 cells were used to measure cell shortening in each heart, and an average was obtained for each value.

Data analysis. All values are shown as means ± SE. Statistical comparison of incidence of arrhythmia and mortality was performed by using Fisher's exact test. For statistical analysis of hemodynamics, two-way ANOVA followed by Dunnett's test was performed. When the F value, calculated by using Bartlett's test was significant, the Kruskal-Wallis nonparametric ANOVA followed by Dunn's test was used. For myocyte experiments, statistical significance was determined with Tukey's or Dunn's test after ANOVA. A P < 0.05 was considered statistically significant. All analyses were performed on the absolute values for the representative parameters.

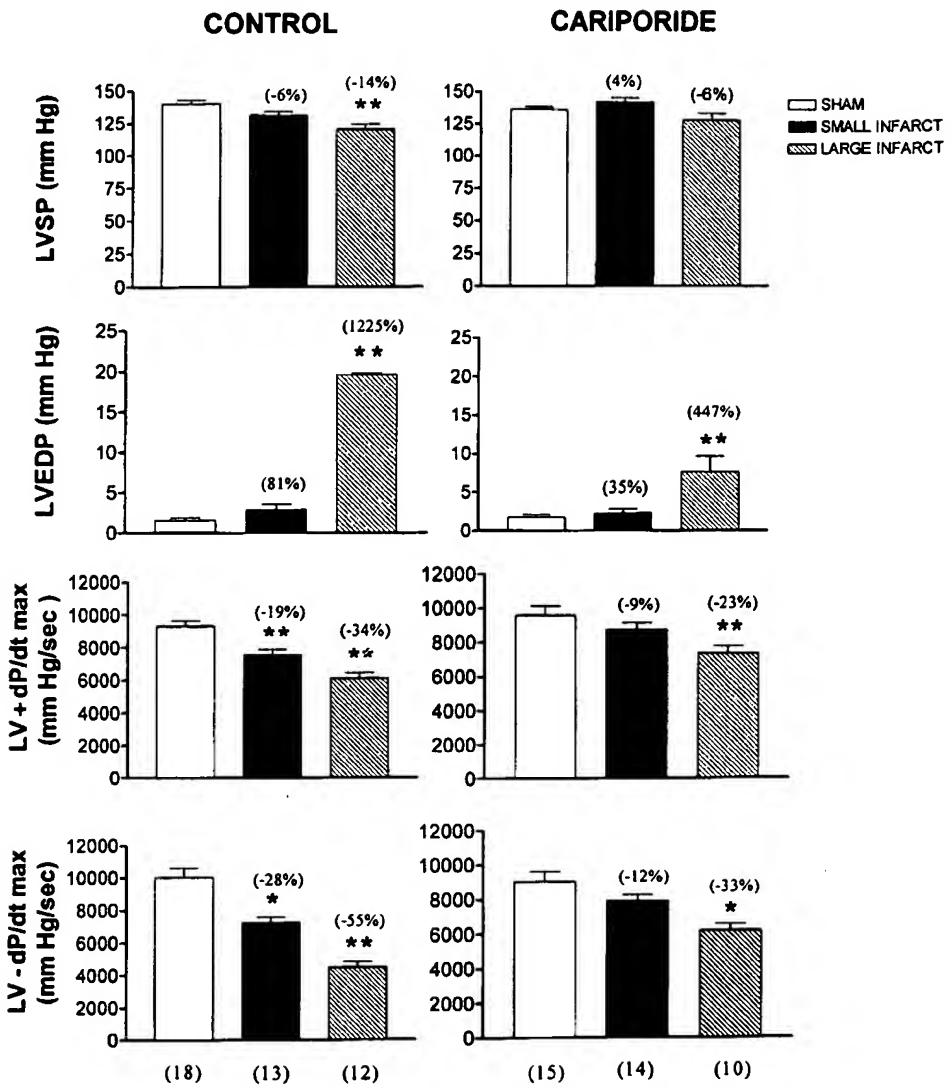

Fig. 1. In vivo hemodynamic characteristics in rats 3 mo after myocardial infarction produced by coronary artery ligation (CAL). Animals were divided according to the degree of infarct sizes as described in METHODS. The cariporide group represents those animals initially administered the sodium/hydrogen exchange (NHE-1)-specific inhibitor cariporide immediately after ligation and then maintained on rat chow containing 3 parts per million cariporide. Control animals were initially administered saline and maintained on an identical diet, although not containing the drug. LVSP, left ventricular systolic pressure; LVEDP, LV end-diastolic pressure; LV $+dP/dt_{max}$, LV maximal increase in pressure over time; LV $-dP/dt_{max}$, LV maximal decrease in pressure over time. $^*P < 0.05$, $^{**}P < 0.01$ from respective sham values. Percentages in parentheses indicate the mean percent change from sham. Numbers in parentheses at the bottom depict the number of animals in each group.

RESULTS

Effect of cariporide treatment on plasma drug levels. Serum cariporide levels averaged 381 ± 44 and 419 ± 67 ng/ml for the sham and CAL groups, respectively.

Table 2. *Mean blood pressure and heart rates in normal and cariporide-treated animals with or without myocardial infarction*

	Sham	Infarcted	
		Small	Large
n			
Normal diet	18	13	12
Cariporide diet	15	14	10
Mean blood pressure, mmHg			
Normal diet	119 ± 4	114 ± 5	105 ± 4
Cariporide diet	117 ± 3	120 ± 4	110 ± 5
Heart rate, beats/min			
Normal diet	379 ± 9	358 ± 8	363 ± 8
Cariporide diet	375 ± 14	376 ± 14	371 ± 9

Values are means ± SE; *n*, number of rats.

Early incidence of ventricular fibrillation and overall incidence of mortality. A major feature of this model is the relatively high incidence of initial ventricular fibrillation; 45% of control animals fibrillated, which was significantly ($P < 0.05$) reduced to 15% in those animals treated with cariporide. Total mortality during the subsequent observation period was 27% in control and 18% in the cariporide-ligated group, although this difference was not significant.

Infarct sizes and body and heart weights. These data are summarized in Table 1. Cariporide had no effect on infarct size in this permanent occlusion model. Body weights were not significantly affected by coronary artery occlusion but tended to be somewhat smaller in the cariporide group.

LV weight was significantly increased in the small infarct size group but not in those animals treated with cariporide. LV weight was not significantly affected in the large infarct size group, although when corrected for body weight, significant effects were observed. RV weight or RV-to-body weight ratios were elevated only

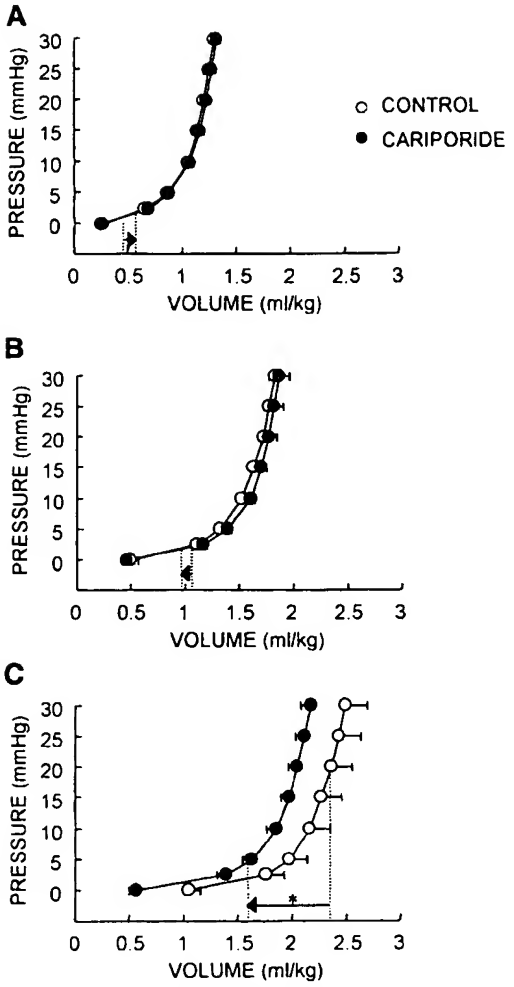

Fig. 2. LV pressure-volume relationships. *A*: sham group, n = 14 control diet, n = 13 cariporide diet; *B*: small infarct group, n = 10 control diet, n = 14 cariporide diet; *C*: large infarct group, n = 14 control diet, n = 8 cariporide diet. Arrows, significant shift of the curve with cariporide. *$P < 0.05$ difference between curves.

in animals exhibiting large infarcts, although this was attenuated significantly by cariporide (Table 1).

Hemodynamic characteristics. Because infarct size greatly influences the hypertrophic remodeling and heart failure processes (12, 13), we grouped the animals (except those used for isolated myocyte studies) into those rats showing small (≤30% of LV) and moderate to large (>30% of LV) infarcts. These data are summarized in Fig. 1 by LV performance. Control animals with small infarcts exhibited moderate hemodynamic changes, although significant attenuations in maximal LV pressure increase over time (LV +dP/dt_{max}) were evident. However, this reduction was not seen in cariporide-treated animals exhibiting identical infarct size. In untreated animals exhibiting large infarcts, LV systolic pressure was reduced by 14% of sham values ($P < 0.05$), whereas this was significantly attenuated by the NHE-1 inhibitor. Moreover, LV +dP/dt_{max} was reduced to a greater degree (34%, $P < 0.05$). However, the magnitude of reduction (23%) was significantly less with drug treatment, although this still represented a significant reduction from sham. A similar profile with respect to LV maximal decrease in pressure over time (−dP/dt_{max}) was observed, including a prevention of significant attenuation in the small infarct group, and marked significant attenuation in animals with large infarcts.

Also evident in Fig. 1, LV end-diastolic pressure (LVEDP) was only moderately affected in animals with small infarcts; however, a marked elevation in LVEDP of 1,225% (from 1.6 ± 0.3 to 19.6 ± 2.4 mmHg, $P < 0.01$) was evident in the large infarct group. This was markedly inhibited by more that 60% compared with the noncariporide group, although these values were still significantly greater than control. Neither heart rates nor blood pressures were affected by any treatment (Table 2).

Pressure-volume relationship. Pressure-volume relationships, an index of LV chamber volume in diastolic stage in vivo under various conditions, is shown in Fig. 2. Infarction resulted in a rightward shift in the pressure volume at the end of the observation period depending on the size of the infarct region. With small infarcts, rightward displacement of the pressure-volume curves was unaffected by cariporide treatment, whereas with large infarcts, a significant attenuation of the rightward shift was observed.

β_1-Adrenergic responses. Heart failure is associated with decreased myocardial response to β_1-adrenergic agonists, and we recently reported that in 1-wk postinfarcted hearts, diminished response to isoproterenol in isolated myocytes from infarcted hearts can be attenuated in animals treated with cariporide. This may suggest that some of the potential beneficial effects of this treatment could involve an attenuation of resistance to catecholamines. Here, we studied whether similar salutary effects of cariporide can be observed in the failing myocardium in vivo 3 mo after infarction by using the β_1-selective agonist dobutamine. As summarized in Fig. 3, dobutamine (0.3–10 μg/kg iv) dose-dependently increased LV +dP/dt_{max} in all groups. However, the amplitude of responses to dobutamine was significantly reduced in animals with large infarcts. Half-maximal effective dose values in sham, small infarct, and large infarct groups maintained on a

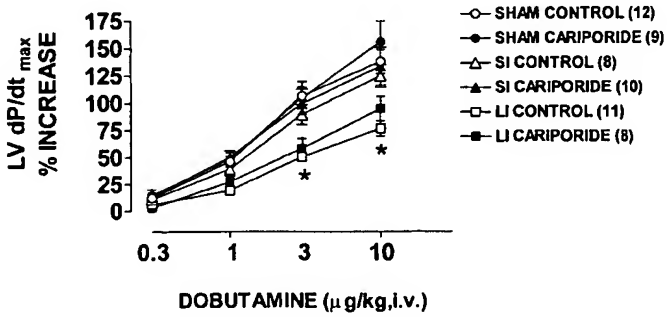

Fig. 3. Dose response to administration of the β_1-adrenoceptor agonist dobutamine on LV dP/dt_{max} after various treatments. SI, small infarct; LI, large infarct. Number of animals is shown in parentheses. *P values for both LI groups were significantly lower from sham.

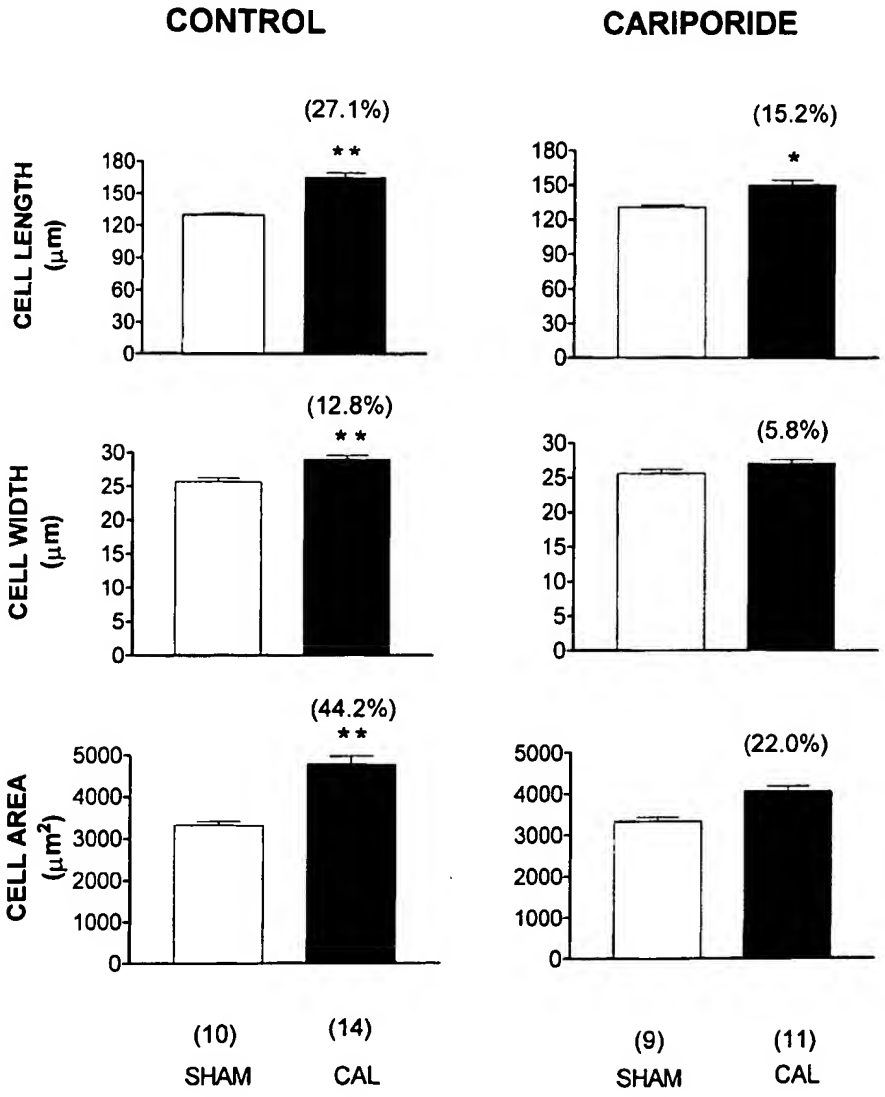

Fig. 4. LV myocyte dimensions after treatment. Numbers in parentheses at bottom depict the number of hearts in each group (mean of 50 cells per heart is 1), and are percent change from respective sham group. *$P < 0.05$, **$P < 0.01$ vs. respective sham groups.

normal diet were 1.0 ± 0.1, 1.3 ± 0.2, and 4.7 ± 1.0 μg/kg ($P < 0.01$), respectively, indicating a significantly depressed response in the latter. Corresponding values in animals given cariporide were 1.0 ± 0.2, 2.2 ± 1.1, and 3.8 ± 1.4 μg/kg ($P < 0.05$), indicating that cariporide had no effect on diminished responsiveness to dobutamine in this particular model.

Characteristics and function of surviving myocytes. To further assess the influence of NHE-1 inhibition, we characterized properties of surviving myocytes by cell dimension and shortening. Because these cells are quiescent, shortening was determined during electrical stimulation. There were no differences in the percentage of rod-shaped viable cells obtained from the various treatment groups, averaging about 80% of the total cell yield. Because it was not possible to isolate myocytes from hearts subjected to infarct size measurements, cells for these studies should be considered as originating from groups exhibiting varied infarct sizes. The data for myocyte dimensions are summarized in Fig. 4. The average myocyte length was significantly increased in control infarcted hearts to about 127% of the respective sham controls. This was attenuated by cariporide to 115%, a value significantly less than in cells from the infarcted group maintained on a control diet. Cell width was significantly increased to 113% of sham values. However, in hearts from cariporide-treated animals, this was almost completely abrogated.

Coronary ligation in untreated rats resulted in a decreased shortening of surviving myocytes of about 27% compared with their respective sham controls. However, myocytes isolated from hearts of cariporide-treated rats showed no diminution in function (Fig. 5).

DISCUSSION

In this study, we presented evidence that NHE-1 inhibition attenuates the adaptive hypertrophic response and congestive heart failure in a rat myocardial infarction model. Our results support and extend the general concept that NHE-1 is an important determi-

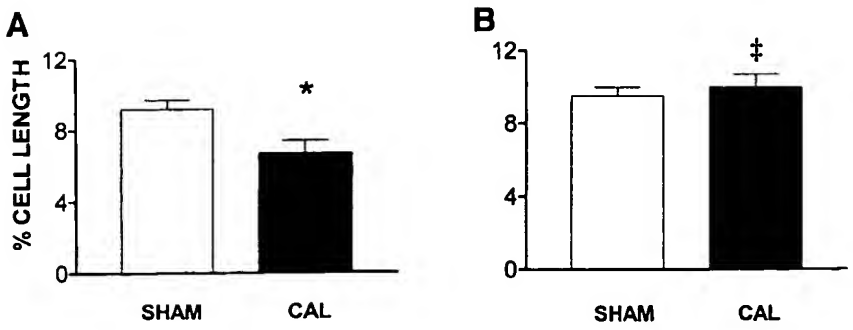

Fig. 5. Ex vivo characteristics of surviving LV myocytes isolated from rats 3 mo after myocardial infarction. Values indicate degree of shortening of LV myocytes isolated after various treatments. *A*: control: $n = 10$ sham, $n = 11$ CAL; *B*: cariporide: $n = 8$ sham, $n = 14$ CAL, respectively. *$P < 0.05$ from respective sham group; ‡$P < 0.01$ from CAL control group.

nant of cell growth in a number of tissues (2, 4, 9, 11, 17, 24). However, its role in postinfarction remodeling, hypertrophy, and subsequent development of heart failure has not been studied in depth. Amiloride, a potassium-sparing diuretic that inhibits numerous ion-regulatory processes including NHE, reduces myocardial fiber size in the 4-wk postinfarcted rat myocardium (9). However, Ruzicka and co-workers (25) recently demonstrated very little improvement in 4-wk postinfarcted hearts from rats treated with amiloride, particularly with regard to LV or RV hypertrophy. This was surprising, in view of the potential importance of NHE-1 in the hypertrophic response, although this may reflect either insufficient inhibition of the antiporter by amiloride or the nonspecific nature of this drug. Other investigators have shown that although NHE-1 inhibition attenuates heart failure, this occurs in concert with infarct size reduction. In cardiac cells, the hypertrophic response to α_1-adrenergic stimulation can be attenuated by NHE inhibition (11, 30). We recently reported (33) that cariporide, a NHE-1 specific inhibitor, significantly blunts the early (7 day) adaptive responses in a postinfarction rat model when animals were placed on the diet 7 days before occlusion, although infarct size was unaffected. From a clinical perspective, we thought it relevant to assess whether this type of approach is effective when cariporide is administered after occlusion and whether any salutary effect persists 3 mo postinfarction. Our results demonstrate that administering a NHE-1 isoform-specific inhibitor of the exchanger limits both the hypertrophic response to infarction and myocardial dysfunction, the latter being particularly evident by marked reduction in the elevation of LVEDP. This reduction in LVEDP may be of particular relevance in view of the importance of diastolic dysfunction in heart failure (18). Although NHE-1 inhibition reduces infarct size in the acutely ischemic myocardium subjected to reperfusion (reviewed in 13), it is important to differentiate the myocardial salvaging effect from a sustained coronary occlusion model without reperfusion used in the present study, where infarct size was not modified, and yet, the heart failure process was attenuated. In addition, these effects were seen in the absence of any effect on blood pressure. Thus a reasonable conclusion from our findings is that NHE-1 inhibition prevents myocardial remodeling in the surviving postinfarcted myocardium. NHE-1 mediates intracellular alkalinization caused by mechanical stretch (2). These investigators (2) proposed that stretch stimulates angiotensin AT_1 and endothelin ET_A receptors which increases phosphoinositide hydrolysis and activates protein kinase C (PKC), resulting in increased NHE-1 activity. However, in the case of endothelin-1, recent evidence suggests NHE-1 activation by this peptide involves mitogen-activated protein (MAP) kinase pathway (21). Irrespective of precise mechanisms underlying NHE-1 activation, these studies suggest NHE-1 inhibition has effects similar to those of endothelin or angiotensin II blockade. However, it is important, and potentially clinically relevant, to note apparent differences with NHE-1 inhibition. For example, angiotensin-converting enzyme inhibitors and endothelin receptor antagonists reduce afterload, which forms the basis for their antihypertensive effects; however, no blood pressure-lowering influence of cariporide was seen in our study, effectively ruling out afterload reduction as a contributing factor.

It is important to note also that cariporide failed to improve the reduced inotropic response to dobutamine. This would suggest that desensitization of the myocardial β_1-adrenergic system in the failing heart is unaffected by cariporide and that the salutary effect of cariporide is unrelated to this pathway.

Although the underlying cellular mechanisms that account for remodeling and the evolution to heart failure are exceedingly complex (3, 14, 29), our data support a role for NHE-1 in the process. The exact mechanisms for NHE-1 involvement, however, remains to be determined, although these mechanisms may involve a permissive effect of NHE-1 activity on protein synthesis, perhaps through pH_i-dependent processes. Thus a potential scenario may involve activation of NHE-1 by various growth factors resulting in hypertrophic responses (reviewed in Ref. 13). We were unable to measure pH_i by using the current protocol, and therefore, the validity of this hypothesis remains uncertain. In view of the multiplicity of pH_i-regulatory mechanisms in the cardiac cell, it is doubtful that intracellular acidosis would be markedly greater in hearts from cariporide-treated animals during sustained occlusion because other mechanisms would compensate for the inability of NHE-1 to remove protons. This is supported by acute ischemia studies where it was observed that pH_i, under conditions of NHE-1 inhibition, generally does not fall lower than

values seen in the absence of NHE-1 blockade (23) or, if pH_i is reduced, the reduction does not occur until late in the ischemic period (16).

It is also important to note that sodium ions are important mediators of cell hypertrophy (5, 10); therefore, the accompanying reduction in sodium entry occurring during NHE-1 inhibition may represent the major basis for salutary effects of cariporide on hypertrophy and heart failure. In a recent study using neonatal rat ventricular myocytes, it was proposed that NHE-1-dependent sodium influx is a major contributor to hypertrophy produced by various agonists, including α_1-adrenergic stimulation, endothelin-1, or phorbol ester by activating various protein kinase C (PKC) isoforms, particularly PKC-δ and PKC-ϵ (10). This concept was reinforced by the ability of PKC inhibitors to reduce the hypertrophic response and by the NHE-1 inhibitor HOE-694 to attenuate both the hypertrophy and PKC activation (10). However, the role of NHE-1 in mediating hypertrophic responses in vitro may also involve more extensive cell-signaling systems. For example, stretch-induced cardiac cell hypertrophy was also associated with Raf-1 and MAP kinase activation with both the hypertrophy and kinase being inhibited by HOE-694, leading the authors to conclude that NHE-1 activates both kinases through a undetermined manner leading to cell growth (30). These authors reported that HOE-694 did not affect upregulation of either Raf-1 or MAP kinases by either endothelin-1 or angiotensin II, although hypertrophic responses were not reported (30). As noted above, in feline papillary muscle, stretch-induced intracellular alkalization was found to be NHE-1-dependent and linked to the activation of both endothelin ET_A and angiotensin II AT_1 receptors via a PKC-dependent process (2). It is clear that unraveling the intracellular processes that mediate NHE-1-dependent cardiac hypertrophy will be challenging in view of the apparent complexity of the process.

In conclusion, our study demonstrates that a NHE-1-selective inhibitor cariporide attenuates the hypertrophic process, and heart failure, in the postinfarcted rat myocardium. This occurs in the absence of infarct size reduction or any effect on blood pressure. Moreover, the resistance toβ_1-adrenoceptor-dependent positive inotropic responses was unaffected by cariporide. When taken together, these findings suggest a direct influence of the drug on remodeling of surviving myocytes, a finding supported by myocyte analysis showing reduced hypertrophy and preservation of ex vivo function. The degree of attenuation of postinfarction responses was, roughly speaking, ~50% compared with values seen in the nontreated group. The failure to completely abrogate the remodeling-heart failure process was not surprising in view of the underlying complexity of postinfarction remodeling, hypertrophy, and heart failure, that is unlikely to be amenable to one therapeutic intervention. Nonetheless, it is possible that a higher dose of cariporide could exert greater beneficial effect, although this needs to be determined. Overall, however, our results suggest that in principle, NHE-1 inhibition represents a desirable approach to reduce the postinfarction heart failure process and could represent an attractive therapeutic approach. It can also be suggested that the benefits of NHE-1 inhibitors could be accentuated when used in combination with other therapies for the treatment of heart failure.

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REFERENCES

1. **Chakrabarti S, Hoque ANE, and Karmazyn M.** A rapid ischemia-induced apoptosis in isolated rat hearts and its attenuation by the sodium/hydrogen exchange inhibitor HOE 642 (cariporide). *J Mol Cell Cardiol* 29: 3169–3174, 1997.
2. **Cingolani HE, Alvarez BV, Ennis IL, and Camillión de Hurtado MC.** Stretch-induced alkalinization of feline papillary muscle: an autocrine-paracrine system. *Circ Res* 83: 775–780, 1998.
3. **Cohn JN.** The management of chronic heart failure. *N Engl J Med* 335: 490–498, 1996.
4. **Dostal DE and KM Baker.** Angiotensin and endothelin: messengers that couple ventricular stretch to the Na^+/H^+ exchanger and cardiac hypertrophy. *Circ Res* 83: 870–873, 1998.
5. **Gu JW, Anand V, Shek EW, Moore MC, Brady AL, Kelly WC, and Adair TH.** Sodium induces hypertrophy of cultured myocardial myoblasts and vascular smooth muscle cells. *Hypertension* 31: 1083–1087, 1998.
6. **Gumina RJ, Buerger E, Eickmeier C, Moore J, Daemmgen J, and Gross GJ.** Inhibition of the Na^+/H^+ exchanger confers greater cardioprotection against 90 min of myocardial ischemia than ischemic preconditioning in dogs. *Circulation* 100: 2519–2526, 1999.
7. **Gumina RJ, Daemmgen J, and Gross GJ.** Inhibition of the Na^+/H^+ exchanger attenuates phase 1b ischemic arrhythmias and reperfusion-induced ventricular fibrillation. *Eur J Pharmacol* 396: 119–124, 2000.
8. **Gunasegaram S, Haworth RS, Hearse DJ, and Avkiran M.** Regulation of sarcolemmal Na^+/H^+ exchanger activity by angiotensin II in adult rat ventricular myocytes: opposing actions via AT_1 vs. AT_2 receptors. *Circ Res* 85: 919–930, 1999.
9. **Hasegawa S, Nakano M, Taniguchi Y, Imai S, Murata K, and Suzuki T.** Effects of Na^+-H^+ exchange blocker amiloride on left ventricular remodeling after anterior myocardial infarction in rats. *Cardiovasc Drugs Ther* 9: 823–826, 1995.
10. **Hayasaki-Kajiwara Y, Kitano Y, Iwasaki T, Shimamura T, Naya N, Iwaki K, and Nakajima M.** Na^+ influx via Na^+/H^+ exchange activates protein kinase C isozymes δ and ϵ in cultured neonatal rat cardiac myocytes. *J Mol Cell Cardiol* 31: 1559–1572, 1999.
11. **Hori M, Nakatsubo N, Kagiya T, Iwai K, Sato H, Iwakura K, Kitabatake A, and Kamada T.** The role of Na^+/H^+ exchange in norepinephrine-induced protein synthesis in neonatal cultured cardiomyocytes. *Jpn Circ J* 54: 535–539, 1990.
12. **Humphreys RA, Haist JV, Chakrabarti S, Feng QP, Arnold JMO, and Karmazyn M.** Orally administered NHE1 inhibitor cariporide reduces acute responses to coronary to occlusion and reperfusion. *Am J Physiol Heart Circ Physiol* 276: H749–H757, 1999.
13. **Karmazyn M, Gan XT, Humphreys RA, Yoshida H, and Kusumoto K.** The myocardial Na^+-H^+ exchange: structure, regulation, and its role in heart disease. *Circ Res* 85: 777–786, 1999.
14. **Katz AM.** The hypertrophic response: programmed cell death. In: *Heart Failure. Pathophysiology, Molecular Biology, and Clinical Management.* Baltimore, MD: Lippincott Williams & Wilkins, 2000, p. 173–226.

15. **Khandoudi N, Ho J, and Karmazyn M.** Role of Na^+-H^+ exchange in mediating effects of endothelin-1 on normal and ischemic/reperfused hearts. *Circ Res* 75: 369–378, 1994.
16. **Koike A, Akita T, Hotta Y, Takeyka K, Kodama I, Murase M, Abe T, and Toyama J.** Protective effects of dimethyl amiloride against postischemic myocardial dysfunction in rabbit hearts: phosphorus 31-nuclear magnetic resonance measurements of intracellular pH and cellular energy. *J Thorac Cardiovasc Surg* 112: 765–775, 1996.
17. **Kranzhofer R, Schirmer J, Schomig A, von Hodenberg E, Pestel E, Metz J, Lang HJ, and Kubler W.** Suppression of neointimal thickening and smooth muscle cell proliferation after arterial injury in the rat by inhibitors of Na^+-H^+ exchange. *Circ Res* 73: 264–268, 1993.
18. **Little WC and Cheng CP.** Diastolic dysfunction. *Cardiol Rev* 6: 231–239, 1998.
19. **Mathur S and Karmazyn M.** Interaction between anesthetics and the sodium-hydrogen exchange inhibitor HOE 642 (cariporde) in ischemic and reperfused rat hearts. *Anesthesiology* 87: 1460–1469, 1997.
20. **Matsui H, Barry WH, Livsey C, and Spitzer KW.** Angiotensin II stimulates sodium-hydrogen exchange in adult rabbit ventricular myocytes. *Cardiovasc Res* 29: 215–221, 1995.
21. **Moor AN and Fliegel L.** Protein kinase-mediated regulation of the Na^+/H^+ exchanger in the rat myocardium by mitogen-activated protein kinase-dependent pathways. *J Biol Chem* 274: 22985–22992, 1999.
22. **Myers ML, Farhangkhoee P, and Karmazyn M.** Hydrogen peroxide induced impairment of postischemic recovery of rat ventricular function is prevented by the sodium-hydrogen exchange inhibitor HOE 642 (cariporide). *Cardiovasc Res* 40: 290–296, 1998.
23. **Navon G, Werrmann JG, Maron RR, and Cohen SM.** ^{31}P NMR and triple quantum filtered ^{23}Na NMR studies of the effects of inhibition of Na^+/H^+ exchange on intracellular sodium and pH in working and ischemic hearts. *Magn Reson Med* 32: 556–564, 1994.
24. **Quinn DA, Dahlberg CG, Bonventre JP, Scheid CR, Honeyman T, Joseph PM, Thompson BT, and Hales CA.** The role of Na^+/H^+ exchange and growth factors in pulmonary artery smooth muscle cell proliferation. *Am J Respir Cell Mol Biol* 14: 139–145, 1996.
25. **Ruzicka M, Yuan B, and Leenen FH.** Blockade of AT_1 receptors and Na^+/H^+ exchanger and LV dysfunction after myocardial infarction in rats. *Am J Physiol Heart Circ Physiol* 277: H610–H616, 1999.
26. **Sadoshima J, Xu Y, Slayter HS, and Izumo S.** Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell* 75: 977–984, 1993.
27. **Silvestre JS, Heymes C, Oubénaïssa A, Robert V, Aupetit-Faisant B, Carayon A, Swynghedauw B, and Delcayre C.** Activation of cardiac aldosterone production in rat myocardial infarction: effects of angiotensin II receptor blockade and role in cardiac fibrosis. *Circulation* 99: 2694–2701, 1999.
28. **Spitznagel H, Chung O, Xia Q, Rossius B, Illner S, Jahnichen G, Sandmann S, Reinecke A, Daemen MJ, and Unger T.** Cardioprotective effects of the Na^+/H^+-exchange inhibitor cariporide in infarct-induced heart failure. *Cardiovasc Res* 46: 102–110, 2000.
29. **Swynghedauw B.** Molecular mechanisms of myocardial remodeling. *Physiol Rev* 79: 215–262, 1999.
30. **Yamazaki T, Komuro I, Kudoh S, Zou Y, Nagai R, Aikawa R, Uozumi H, and Yazaki Y.** Role of ion channels and exchangers in mechanical stretch-induced cardiomyocyte hypertrophy. *Circ Res* 82: 430–437, 1998.
31. **Yamazaki T, Komuro I, Kudoh S, Zou Y, Shiojima I, Hiro Y, Mizuno T, Maemura K, Kurihara H, Aikawa R, Takano H, and Yazaki Y.** Endothelin-1 is involved in mechanical stress-induced cardiomyocyte hypertrophy. *J Biol Chem* 271: 3221–3228, 1996.
32. **Yokoyama H, Yasutake M, and Avkiran M.** α_1-adrenergic stimulation of sarcolemmal Na+-H+ exchanger activity in rat ventricular myocytes: evidence for selective mediation by the α_{1A}-adrenoceptor subtype. *Circ Res* 82: 1078–1085, 1998.
33. **Yoshida H and Karmazyn M.** Na^+/H^+ exchange inhibition attenuates hypertrophy and heart failure in 1-wk postinfarction rat myocardium. *Am J Physiol Heart Circ Physiol* 278: H300–H3004, 2000.